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A COMPARISON OF *IN SITU* AND *IN VITRO* RESPONSES OF CRUSTACEAN HEARTS TO HYPOXIA [1]

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Although crustacean cardiac physiology has been extensively researched, there remain several aspects which have scarcely been investigated. One such area has been the response of the crustacean heart to deficiencies in oxygen.

An early indication of the cardiac response to hypoxia was noted by Burger and Smythe (1953) in the lobster *Homarus americanus*. It was observed that the heart of this animal slowed its rate of beating when the lobster was out of the water. In this situation the gill filaments would be collapsed and the effective exchange area reduced, thereby placing the animal in an hypoxic situation. Larimer (1962, 1964a, 1964b), in a series of studies on the crayfish *Procambarus simulans*, found that these animals demonstrate a marked bradycardia when the oxygen is driven from the water containing them. Thompson and Pritchard (1969) subjected the burrowing shrimp *Callianassa californiensis* to hypoxia and noted a significant reduction of heart rate at very low oxygen concentrations.

In the present investigation the response to hypoxia of the heart of the crab *Cancer magister* (Dana) will be described. Previous investigations of these responses have been limited to the response of the *in situ* heart. This report will also deal with the response of the isolated heart to hypoxia. The effect of lowered oxygen concentrations on amplitude or magnitude of contraction is also examined.

Methods and Materials

This study was carried out at the Oregon State University Marine Science Center at Newport, Oregon and at the main campus in Corvallis.

Adult specimens of *Cancer magister* ranging in carapace width from 10 to 18 cm were collected from Yaquina Bay and maintained at 12° C.

All experiments were performed at 12 ± 1° C which approximates the temperature in the area from which the animals were collected.

Recording of heart rate and amplitude of contraction was achieved by the use of a Narco Bio-Systems physiograph in conjunction with a Narco Type B photoelectric force transducer-type myograph.

Oxygen concentration in the experimental containers was manipulated by bubbling nitrogen or air directly into the water or perfusion solution (in the case of the isolated hearts). A Yellow Springs Instrument Company Model 54 oxygen meter and Model 5420 oxygen probe were used to moniter the oxygen concentration in the testing bath.

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Animals were prepared for *in situ* recording by inducing autotomy of their legs and then tied to a restraining board. Limb autotomy seemed to have no effect on the pattern of the recorded heart beat, as demonstrated by recordings of the heart beat of a few animals with intact legs. After the removal of a small piece of carapace overlying the heart, a bent pin was inserted into the still intact hypodermis and connected by a thread to the myograph. The oxygen probe and air stones were then positioned in the bath and the container was covered to restrict the entry of air.

The preparation for the *in vitro* heart beat recordings involved a modification of a method described by Welsh and Smith (1960). The pericardial cavity was carefully exposed and ligatures were tied around the posterior lateral ligaments of the heart. These later served as a convenient means of handling the heart. Next, the abdominal artery was cannulated with a piece of suitable sized polyethylene tubing. Finally the anterior arteries were ligatured and the heart was removed to the recording chamber and attached to the myograph. The recording chamber consisted of a $4 \times 4 \times 6$ inch plastic refrigerator container equipped with air stones and an oxygen probe. The bottom of the chamber was pierced by a tube through which the perfusion solution passed and to which the cannula was attached.

After mounting the heart in the chamber, the rate of perfusion could be controlled by means of a Teflon needle valve. Shortly after initiating perfusion the heart resumed its rhythmic beat with a steady rate and amplitude. Before a preparation was judged usable it had to meet the following criteria: display of reasonably steady rate and amplitude; recovery to near normal rate and amplitude after hypoxic stress; and response to changes in perfusion rate with corresponding changes in rate and amplitude. An increase in perfusion rate has been shown to result in an increase in rate and amplitude for the heart of *Maia squinado* (Izquierdo, 1931).

All hearts were perfused with a solution identical to that used by Davenport (1941).

The same protocol was used for both *in situ* and isolated hearts in determining the response to hypoxia. After the recording instruments were attached the animal was allowed to stabilize at least ten minutes and the oxygen concentration of the water was recorded. Nitrogen was then passed through the water for a short period. After the heart beat and oxygen concentration had again stabilized the procedure was repeated. This was done until the oxygen concentration had decreased to below 1 mg O_2 per liter of sea water. Air was then re-introduced into the bath and the heart beat was allowed to return to normal. During the recovery period the oxygen concentration was periodically recorded in order to evaluate the latency of the response to oxygen.

Results

The heart rates of the crabs were found to vary somewhat from animal to animal but were fairly consistent from one experiment to the next for a given animal. The mean rate for *in situ* hearts when beating at maximum was 79 beats per minute with a range of 72 to 92. The mean rate for isolated hearts at maximum was 54 beats per minute with a range of 37 to 81.

Since this investigation is primarily concerned with relative values the data are expressed as a per cent of maximum rate. To prevent the possibility of random accelerations of short duration from biasing the data, the maximum rate had to be sustained for a period of at least three minutes to be considered as valid. The resultant values are best termed relative rate.

The amplitude recorded by the myograph provides a rough index of the strength of contraction of the hearts. This is quantifiable in terms of relative amplitude. In some of the recordings amplitude varied slightly from contraction to contraction but was maintained at an overall consistent level. This was especially so in the case of the *in situ* hearts. To determine the relative amplitude under these conditions, the heights of ten consecutive peaks, chosen at random, were averaged. The myographic recording of the amplitude of contraction in *in situ* hearts is complicated by the complex attachments of the heart, both within

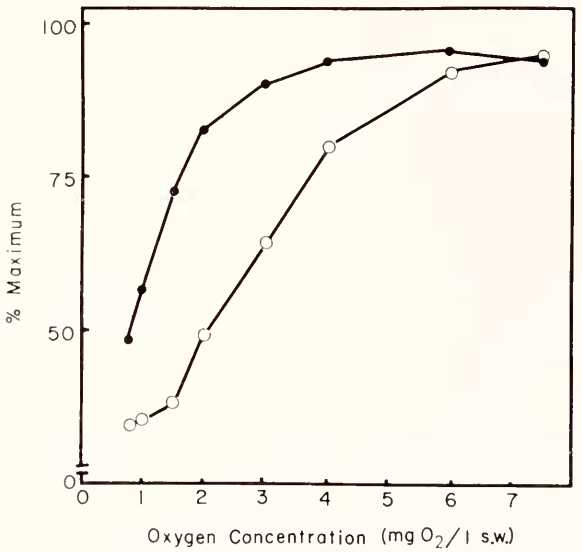

FIGURE 1. Relationship between heart rate (●) and amplitude (○) and oxygen concentration for *in situ* hearts. Each curve represents the results of eight experiments in which rate and amplitude were recorded simultaneously. The rates and amplitudes are expressed in terms of per cent of maximum while the oxygen concentration is in mg O_2/l sea water. The standard errors ranged from 0.8% to 6.0% for rate and from 2.0% to 8.1% for amplitude.

the pericardial cavity and externally with the myograph. The attachment of the heart to the myograph in the *in situ* state is, of necessity, indirect due to the need to preserve integrity of the open circulatory system. To accomplish this the pin connected to the myograph was inserted in the hypodermis which, in turn, was indirectly connected to the heart. This left the open circulatory system intact but resulted in a complex transmission of the heart's action to the myograph. This did not affect the recording of rate but may have significantly altered the pattern of recorded amplitude. With the isolated hearts the myograph was connected directly to the hearts themselves. Since the amplitude response of the isolated hearts was

similar in nature to that of the *in situ* hearts the greater variability of the latter may have been due to the indirect means of recording.

A persistent feature of many *in situ* recordings was the occasional appearance of a transient cardiac arrest. This was observed to occur both in diastole (Fig. 3a) and in systole and was often seen either accompanying or just preceding limb or limb stub movement. Similar cardiac inhibition has been reported in *Asellus aquaticus,* an isopod (Needham, 1954), *Panulirus argus,* a lobster (Maynard, 1960) and *Procambarus clarkii,* a crayfish (Larimer and Tindel, 1966).

The responses of both *in situ* and isolated hearts to hypoxia were marked bradycardia and depression of amplitude. Although the responses were similar in most respects, certain differences were apparent. Figure 1 depicts the response of the *in situ* hearts to hypoxia and it may be seen that as oxygen concentration fell below 1 mg O_2/l both rate and amplitude decreased to less than 50 per cent of the values in air-saturated water. The pattern of this decrease, however, is different for rate and amplitude. While the decrease in rate is clearly hyperbolic, with the most significant decrease occurring between 1 and 2 mg O_2/l, the decrease in amplitude tends more toward linearity in this range of oxygen concentrations. The patterns of decrease in rate and amplitude of isolated hearts, however, are very much alike, both tending toward a hyperbolic relationship (Fig. 2). It is felt that the

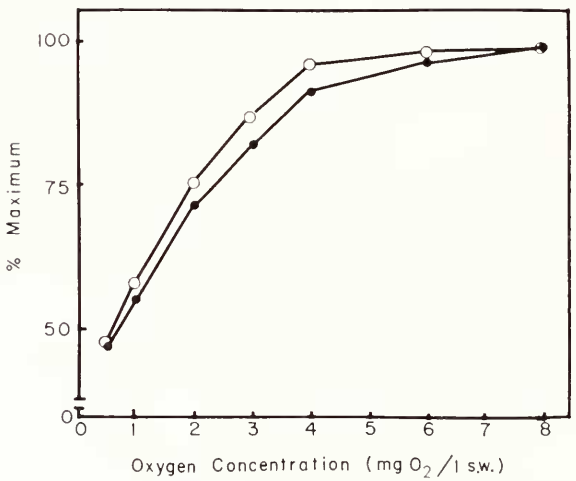

FIGURE 2. Relationship between heart rate (●) and amplitude (O) and oxygen concentration for isolated hearts. Each curve represents the results of six experiments in which rate and amplitude were recorded simultaneously. The rates and amplitudes are expressed in terms of per cent of maximum while the oxygen concentration is in mg O_2/l sea water. The standard errors ranged from 0.6% to 8.0% for rate and from 0.6% to 5.4% for amplitude.

indirect means of recording *in situ* heart movements is responsible for the apparently anomalous pattern of *in situ* amplitude.

The time course of the recovery of the hearts to near the maximum rate upon readmission of oxygen to the water after hypoxic stress differed greatly in *in situ* and isolated hearts. Nineteen experiments were performed and in each case the

response of the *in situ* hearts to oxygen was much more rapid than was the response of the isolated hearts. The mean recovery time for *in situ* hearts was 22 seconds while the mean recovery time for isolated hearts was 127 seconds. These are significantly different at the 0.001 level (t-test). The mean rates of increase

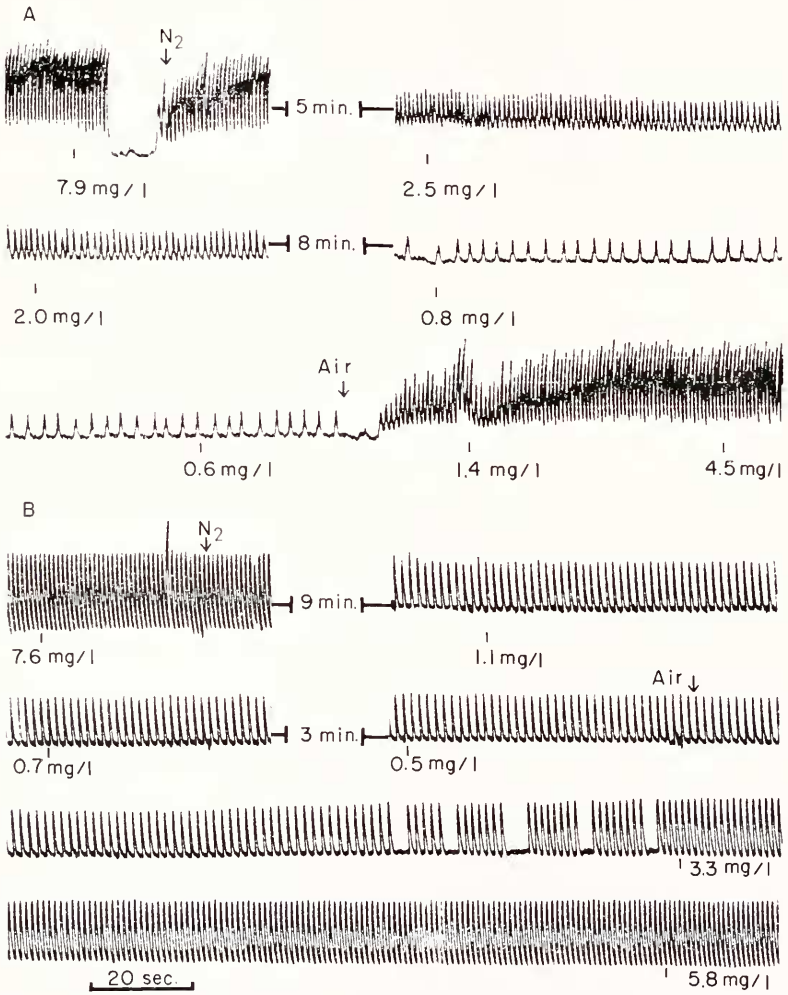

FIGURE 3. Recordings of typical *in situ* (A) and isolated (B) heart beats. The records are continuous except where breaks are indicated. Oxygen concentration at various points is given in mg O_2/l sea water.

of oxygen concentration were roughly similar in the two situations being 1.82 mg O_2/l/min for *in situ* and 1.24 mg O_2/l/min for isolated preparations. Typical recordings showing these responses are given in Figure 3.

As a precaution against the possibility that the response of the *in situ* hearts to aeration of hypoxic water may have been a mechanoreceptive one, associated with

the turbulence caused by aeration, several crabs were subjected to sudden vigorous nitrogen bubbling while under hypoxic stress (Fig. 4). In no case was there a duplication of the aeration response.

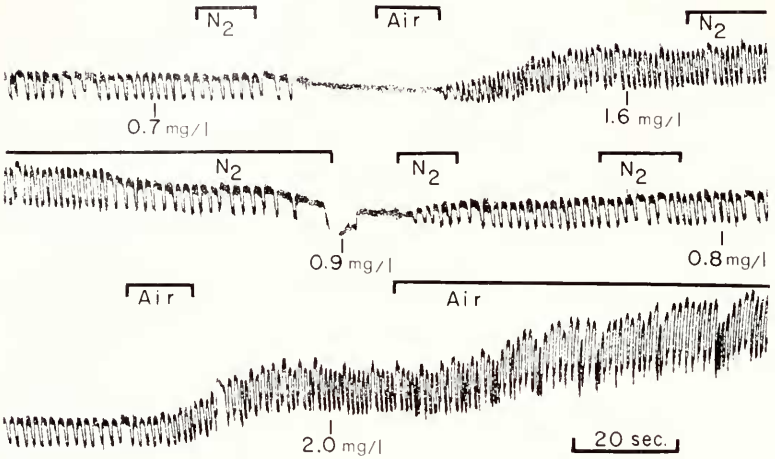

FIGURE 4. Continuous recording showing the failure of the *in situ* heart to respond to turbulence caused by vigorous nitrogen bubbling. The duration of aeration and nigration bubbling is shown above recording while oxygen concentration of the water is indicated below.

DISCUSSION

The variation in the maximum heart rate probably results from a number of factors. Since the animals were held for varying lengths of time without feeding, their nutritional states might have varied. More importantly, different levels of "trauma" following preparation of the animals undoubtedly had some effect on the level of heart rate and amplitude. It is felt, however, that since these parameters were quite stable within a given preparation, and since relative rates and amplitudes were used, the variability does not significantly alter the interpretations of the data.

A comparison of the mean maximum heart rates of the isolated and *in situ* hearts shows that the latter beat much more rapidly (79 min *vs.* 54/min). The difference may be due to the absence of the cardio-acceleratory nerves and possibly the pericardial organs in the isolated preparations. The pericardial organs are neurosecretory elements which have a pronounced cardio-excitatory effect (Cooke, 1964).

The bradycardia resulting from hypoxia confirms that observed in other crustaceans investigated. These include *Procambarus simulans* (Larimer, 1962, 1964a, 1964b) and *Callainassa californiensis* (Thompson and Pritchard, 1969).

The significance of the hypoxia-induced bradycardia is not clear. Larimer (1962) felt that it might be due to a high sensitivity of the heart muscle to lowered oxygen concentration. Subsequent work (Larimer, 1964a), however, indicates that this may not be the case, at least for *P. simulans*, as the per cent extraction of oxygen from the respiratory stream increased during hypoxia. To explain this

Larimer has hypothesized an increased rate of circulation, facilitated by an increased stroke volume and decreased peripheral resistance during bradycardia. A similar increased per cent extraction of oxygen has been reported for the lobster *Homarus vulgaris* during hypoxia (Thomas, 1954) and circulatory responses were also postulated as an explanation.

The increased circulation rate at low concentrations hypothesized by Larimer (1964a) might be advantageous for an animal such as *P. simulans*, whose respiratory pigment has a high affinity for oxygen. Larimer and Gold (1961) report a P_{50} of 3.5 mm Hg for this crayfish. *Cancer magister*, however, has a low oxygen affinity hemocyanin with a P_{50} of 19.6 mm Hg at 10° C (Johansen, Lenfant and Mecklenburg, 1970). *Callianassa californiensis* also has a high oxygen affinity hemocyanin with a P_{50} of 3–4 mm Hg at 10° (Miller and Pritchard, unpublished data), and shows a bradycardia at low oxygen concentrations (Thompson and Prithcard, 1969). Data regarding changes in per cent extraction of oxygen for *Cancer magister* during hypoxia are not available. However, Johansen *et al.* (1970) report an increase in the gradient between exhaled water and arterial blood oxygen during partial hypoxia which might reflect a decreased per cent extraction of oxygen from the respiratory stream. This, however might also be explained by a possible increased ventilation in these animals during hypoxia (Johansen *et al.*, 1970; Stiffler, 1970).

Cancer magister is capable of regulating its metabolic rate over a wide range of oxygen concentrations (Johansen *et al.*, 1970). It is noteworthy that the lower limits of this regulation correspond roughly to the range of oxygen concentrations in which the decrease in heart rate and amplitude, reported here, are most apparent. Regulation of metabolic rate over a wide range has also been observed in the shrimp *C. californiensis* (Thompson and Pritchard, 1969). Bradycardia in this species also occurred at oxygen concentrations roughly corresponding to the critical oxygen concentration at which metabolic rate began to decrease. These authors suggested that the maintenance of a constant heart rate aids this animal in regulating its oxygen consumption rate.

The decrease in amplitude of contraction observed in this study is difficult to interpret. The fact that it occurred consistently in isolated hearts as well as *in situ* hearts would seem to indicate that it is not an artifact of pin placement in the latter case. Although the amplitude of contraction is certainly not a reliable index of cardiac output, changes in this parameter may roughly indicate changes in stroke volume. If this is the case the increased stroke volume hypothesized by Larimer (1964a) for the crayfish does not appear to be operating in the case of *Cancer magister*. What may be happening is that as the oxygen concentration decreases below the range where the animals regulate metabolic rate and oxygen consumption declines, the circulation rate also decreases. The fact that the isolated hearts showed a bradycardia under hypoxic conditions indicates that the heart muscle may be sensitive to oxygen deprivation in this animal.

All of this suggests that an increased rate of circulation during hypoxic stress as proposed by Larimer (1964a) for crayfish is not a generalized response for decapod crustaceans. The present data, as well as that of Johansen *et al.* (1970) and Thompson and Pritchard (1969), suggest that the bradycardia may be accompanied by a decrease in circulatory rate in at least some decapods. In any case

the interactions involved in gas exchange cannot be fully assessed for *Cancer magister* until measurements of cardiac output and per cent extraction of oxygen from the respiratory stream have been made under conditions of varying oxygen concentration.

The response of the isolated hearts to hypoxia was quite similar to that of the *in situ* hearts. The correlation between changes in amplitude and rate was much closer in the case of the isolated hearts, however. For the *in situ* hearts the relationship between heart rate and oxygen concentration was clearly hyperbolic, whereas the relationship between amplitude and oxygen concentration tended more toward linearity (Fig. 1). This might be explained by the indirect connection between the heart and the myograph discussed above. With the isolated hearts, both functions tended toward a hyperbolic relationship (Fig. 2). This might be expected with the more direct means of recording the actions of the isolated hearts.

One of the more interesting differences between the isolated and *in situ* hearts' responses lies in their respective patterns of recovery. While the *in situ* hearts recovered quite rapidly, within a few seconds of aeration of hypoxic water, the isolated hearts were quite slow to recover, consistently taking several minutes to do so (Fig. 3a and 3b). As there is no way of instantaneously restoring the oxygen to the water the dead time involved in aeration may be significant.

A possible explanation of the discrepancy in these latencies is that the *in situ* heart remained under the control of the animal's nervous system. This would be suggestive of a receptor or receptors sensitive to oxygen. Such hypothetical receptors have been implicated in the responses of other arthropods to oxygen (Waterman and Travis, 1953; Larimer, 1964a; Farley and Case, 1969; Gamble, 1971). The possibility that the response might be due to the turbulence associated with aeration can be ruled out as vigorous bubbling of nitrogen, initiated suddenly in still, hypoxic water, did not elicit the response (Fig. 4).

Although the idea of oxygen receptors is an intriguing one, until such receptors are found and action potentials recorded from their nerves at varying oxygen concentrations, their existence must remain highly speculative.

Summary

1. Heart rate and amplitude were recorded for both *in situ* and isolated hearts of *Cancer magister* exposed to lowered oxygen concentrations.

2. Both rate and amplitude declined markedly as the oxygen was driven from the water. This occurred in both *in situ* and isolated hearts.

3. The recovery of *in situ* hearts to near normal rate and amplitude occurred quite rapidly upon the readmission of oxygen to the water.

4. Isolated hearts recovered very slowly to aeration of hypoxic water.

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